MOLECULAR AND CELLULAR BIOLOGY, May 1996, p. 2394–2401 Vol. 16, No. 5
0270-7306/96/$04.00+0

Helix-Loop-Helix Proteins LYL1 and E2a Form Heterodimeric Complexes with Distinctive DNA-Binding Properties in Hematolymphoid Cells

ALISON MIYAMOTO, XIANGMIN CUI, LOUIE NAUMOVSKI, AND MICHAEL L. CLEARY*

Laboratory of Experimental Pathology, Department of Pathology, Stanford University School of Medicine, Stanford, California 94305

Received 4 January 1996/Returned for modification 6 February 1996/Accepted 22 February 1996

LYL1 is a basic helix-loop-helix (HLH) protein that was originally discovered because of its translocation into the β T-cell receptor locus in an acute lymphoblastic leukemia. LYL1 is expressed in many hematolymphoid cells, with the notable exceptions of thymocytes and T cells. Using the yeast two-hybrid system to screen a cDNA library constructed from B cells, we identified the E-box-binding proteins E12 and E47 as potential lymphoid dimerization partners for LYL1. The interaction of LYL1 with E2a proteins was further characterized in vitro and shown to require the HLH motifs of both proteins. Immunoprecipitation analyses showed that in T-ALL and other cell lines, endogenous LYL1 exists in a complex with E2a proteins. A preferred DNA-binding sequence, 5′-AACAGATG(T/g)T-3′, for the LYL1-E2a heterodimer was determined by PCR-assisted site selection. Endogenous protein complexes containing both LYL1 and E2a bound this sequence in various LYL1-expressing cell lines and could distinguish between the LYL1 consensus and μE2 sites. These data demonstrate that E2a proteins serve as dimerization partners for the basic HLH protein LYL1 to form complexes with distinctive DNA-binding properties and support the hypothesis that the leukemic properties of the LYL and TAL subfamily of HLH proteins could be mediated by recognition of a common set of target genes as heterodimeric complexes with class I HLH proteins.

The *LYL1* gene was originally identified at the breakpoint of a t(7;19) chromosomal translocation carried by a T-cell acute lymphocytic leukemia (T-ALL) (30). The juxtaposition of *LYL1* on chromosome 19 with the T-cell receptor β gene locus on chromosome 7 activated ectopic expression of LYL1, which is not normally expressed in T-lineage cells (28, 47). Two other proteins, TAL1 (SCL) and TAL2, with partial sequence similarities to LYL1 are also ectopically activated by chromosomal translocations in T-ALL cells (6, 9, 51). On the basis of their sequence homologies and similar associations with chromosomal translocations in T-lineage leukemias, the LYL and TAL proteins may represent a structurally and functionally distinct subfamily of proteins involved in a subset of acute leukemias (2).

The LYL1 protein contains a motif known as the basic helix-loop-helix (bHLH) domain. The bHLH motif is well conserved between species, with representative bHLH proteins involved in maize pigmentation (*B* gene), yeast basal transcription (TFIIIC), *Drosophila* neurogenesis (achaete scute), and mammalian myogenesis (MyoD, myogenin, myf-5, and herculin). The bHLH motif was originally hypothesized to form a structure that mediated dimerization through the HLH domains and DNA binding through the basic regions of the dimerized proteins. The crystal structures of two different HLH homodimers in complex with DNA have been solved and have borne out this prediction (13, 15). The binding sites for bHLH proteins adhere to the consensus NNCANNTGNN, otherwise known as E boxes (26). These sites actually represent two half sites, each bound by one of the proteins in the dimer complex, with the underlined portion constituting the "core" sequence contacted by the basic regions.

HLH proteins have been classified into two groups. Class I proteins are widely expressed and can homodimerize as well as heterodimerize with other bHLH proteins. Class II proteins are expressed in a tissue-specific manner and generally do not homodimerize but prefer to form heterodimeric complexes with class I proteins (33, 34). Mammalian class I proteins include the *E2a* gene products (E12, E47, and ITF1), E2-2, and HEB (19, 24, 33). Homodimeric complexes of class I proteins have been observed in different cell types and may be functionally important (3, 25, 41). However, other than a role in immunoglobulin heavy-chain and CD4 transcription (41, 42), homodimeric complexes of class I bHLH proteins have been implicated in relatively few biological processes, in spite of the generalized expression of class I HLH proteins in many cell types. A more prevalent functional role for these proteins appears to involve the formation of heterodimers with class II HLH proteins. The best studied of the I-II heterodimers is E2a-MyoD. Although E2a homodimers can bind in vitro to the same E box sites as E2a-MyoD heterodimers, only the heterodimers containing MyoD are capable of inducing transcription of muscle-specific genes (11, 50). Thus, class II HLH proteins appear to be important functional components of an enhancer-binding regulatory network of transcription factors.

Within the LYL1, TAL1, and TAL2 subfamily, a role for TAL1-E2a in erythroid development has recently been described. Targeted gene disruption of TAL1 blocked embryonic erythrocyte formation (37, 44). Forced expression of TAL1 increased the amount of spontaneous differentiation of erythroid precursors, and endogenous TAL1-E2a complexes have been detected in erythroid cells (1, 46). TAL1-E2a and TAL2-E2a complexes have also been shown to bind in a site-specific manner to DNA in vitro (20, 52), and TAL1-E2a complexes have also been observed in leukemic T cells and a myeloid cell line (22, 48). These findings suggest a role for TAL-E2a complexes in the differentiation of some hematopoietic lineages,

* Corresponding author. Phone: (415) 723-5471. Fax: (415) 725-6902.

perhaps similar to the role of MyoD-related proteins in myogenesis.

The present studies were conducted to determine the biochemical properties of LYL1 in comparison with other HLH proteins. The HLH motif of LYL1 was found to mediate heterologous interactions with the E2a group of HLH proteins both in vitro and in several cell lines representative of leukemic T cells, promonocytes, and pre-B lymphocytes. The heterodimeric LYL1-E2a complexes in these cells displayed a DNA-binding specificity distinct from that of E2a homodimers but similar to that of TAL1-E2a heterodimers. These observations demonstrate a functional similarity for the oncogenic LYL1 and TAL subfamily of HLH proteins and support the hypothesis that their leukemic properties could be mediated by recognition of a common set of target genes as heterodimeric complexes with class I HLH proteins.

MATERIALS AND METHODS

Yeast two-hybrid screening. An improved two-hybrid system was used to isolate human genes encoding LYL1-interacting proteins (18). An *Eco*RI-*Bam*HI fragment of *LYL1* cDNA (560 bp encoding amino acids 1 to 226, including the HLH region [construct 1] [30]) was cloned into the *Nco*I and *Bam*HI sites of pAS1 (12) by using synthetic linkers to facilitate maintenance of the open reading frame. Y153 was transformed to Trp prototrophy with pAS1-LYI, and a single colony was grown in Trp-deficient medium. The predicted fusion protein (approximately 44 kDa, consisting of GAL4 amino acids 1 to 147, the hemagglutinin (HA) epitope, a 13-amino-acid linker, and LYL1 amino acids 1 to 126) was detected in crude lysates of transformed yeast (*Saccharomyces cerevisiae*) cells by Western blotting (immunoblotting) using an anti-HA antibody (BAbCo, Richmond, Calif.).

To use an improved two-hybrid screening procedure, an *Nco*I-*Sal*I fragment from pAS1-LYL was cloned into pAS1-CYH2 and transformed into Y190. For library screening, Y190 cells harboring pAS1-CYH2-LYL were transformed with library DNA in the pACT vector (12) by using total yeast RNA as the carrier. The transformation mix was plated on medium lacking tryptophan, leucine, and histidine but including 50 mM 3-aminotriazole (Sigma, St. Louis, Mo.) and incubated at 30°C for 3 to 5 days. His^+ colonies were screened for β-galactosidase activity by a filter lift assay (8). Colonies that produced positive signals were tested in yeast strains for specificity after curing the pAS1-CYH2-LYL plasmid and mating to Y187 tester strains carrying various pAS1 plasmids expressing GAL4 fusion proteins, including GAL4-lamin (5). The resultant diploids were assessed for β-galactosidase activity. Those specific for LYL1 were recovered from yeast cells, transformed into bacteria by electroporation, and isolated for sequence analyses.

Sequence analyses. pACT library plasmids resulting from the yeast two-hybrid screen were sequenced by using dideoxynucleoside triphosphates and Sequenase 2.0 according to the manufacturer's (U.S. Biochemicals, Cleveland, Ohio) specifications. Sequence analyses and similarity searches were performed by using the BLAST algorithm on the EMBL and GenBank databases.

In vitro transcription and translation. Circular plasmid DNAs were transcribed and translated by using the TnT coupled reticulocyte lysate system (Promega, Madison, Wis.) as instructed by the manufacturer. For coimmunoprecipitation experiments, lysates were either cotranslated in the TnT system or translated separately and then mixed together for 20 min at 37°C before the addition of antibody. Immunoprecipitation analysis was conducted with the appropriate rabbit or mouse antiserum. Immunoprecipitated proteins were electrophoresed on a sodium dodecyl sulfate–12% polyacrylamide gel electrophoresis (SDS-PAGE) gel, and the gel was fixed in a solution of 25% methanol and 10% acetic acid. Dried gels were evaluated on a PhosphorImager by using ImageQuant software (Molecular Dynamics, Sunnyvale, Calif.). A LYL1 mutant lacking the HLH region (amino acid residues 154 to 207) was constructed by deleting the *Hin*cII-*Sma*I fragment from LYL1. The N-terminal truncation of LYL1, pSPLYLΔ2-128, was constructed by PCR and inserted into the *Hin*dIII-*Eco*RI site of pSP64 (Promega). This construct was sequenced and found to contain no PCR-induced mutations. The LYL1-HA-tagged cDNA was constructed by replacing the LYL1 stop codon with an *Eco*RI site by PCR. The HA tag was then cloned into the *Eco*RI site. The E2a mutant lacking the bHLH domain was previously described (32).

Cell extracts, immunoprecipitations, and Western blot analyses. For Western blotting, 10^7 cells were harvested in log phase and pelleted by centrifugation. Cells were washed once with phosphate-buffered saline (PBS). The cell pellet was resuspended in 250 μl of Laemmli SDS buffer (2% SDS, 100 mM dithiothreitol, 50 mM Tris-HCl [pH 6.8], 10% glycerol, 0.1% bromophenol blue) and boiled for 3 min. Chromosomal DNA was sheared by passing the lysate through a 25-gauge needle five times. Cell debris was pelleted by centrifugation, and the supernatant was stored at −70°C. For immunoprecipitations employing hematolymphoid cells, 10^7 cells were pelleted and washed once in PBS. Cells were resuspended in 1 ml of 1% Nonidet P-40 in PBS and lysed on ice for 30 min. Nuclei were lysed by passage through a 25-gauge needle five times, and cell debris was removed by centrifugation. For COS cell immunoprecipitations, transfected cells from a 100-mm-diameter tissue culture dish were scraped off in PBS and treated as described above for hematolymphoid cells. Immunoprecipitations of cell extracts were performed for 1 h on ice with 1 μl of either preimmune or anti-LYL1 polyclonal rabbit antiserum. Immune complexes were recovered on protein A-Sepharose beads (Pharmacia, Piscataway, N.J.) for 1 h at 4°C with gentle rolling. The Sepharose beads were pelleted, washed four times with lysis buffer, and resuspended in Laemmli SDS gel loading buffer. Samples were boiled for 5 min prior to electrophoresis through SDS–12% PAGE gels. Gels were transferred to nitrocellulose (Schleicher & Schuell, Keene, N.H.) on a semidry electroblotting apparatus according to the manufacturer's (Sartorius, Göttingen, Germany) instructions in transfer buffer (48 mM Tris, 39 mM glycine, 0.037% SDS, 20% methanol) for 1 h at 100 mA. The filters were stained with Ponceau S (Sigma) to verify even transfers of proteins. The membrane was blocked overnight at 4°C in 5% nonfat dry milk–0.1% Tween 20 in PBS (Blotto). Blocking solution was washed off the membrane with three changes of PBS-T (0.1% Tween 20 in PBS). Western blotting with the Yae antibody, specific for E2a proteins, was performed as previously described (25), except that goat anti-mouse immunoglobulin G1 antibody conjugated to horseradish peroxidase (Boehringer Mannheim, Indianapolis, Ind.) was used as the secondary antibody. Anti-LYL1 Western analysis was conducted with affinity-purified anti-LYL1 antibody at a 1:10 dilution in Blotto solution for 90 min at room temperature. Filters were washed three times in PBS-T. Goat anti-rabbit horseradish peroxidase-conjugated antibody (Kirkegaard and Perry Laboratories, Gaithersburg, Md.) at a 1:1,000 dilution in Blotto solution was incubated with the filters for 30 min at room temperature. After the filters were washed three times in PBS-T, ECL reagents (Amersham, Arlington Heights, Ill.) were used for detection.

COS cell transfections. MO-COS cells were plated at 10^6 cells per 100-mm-diameter plate in Dulbecco modified Eagle medium plus 10% fetal calf serum. After 16 to 20 h, 25 μg of expression plasmid(s) and 5 μg of pRSV-luc were mixed and the volume was brought up to 450 μl. A 60-μl volume of 2 M $CaCl_2$ was added, and the mixture was incubated at room temperature for 15 min. The DNA solution was added dropwise with a Pasteur pipette to 500 μl of an ice-cold 2× Hanks balanced salt solution and kept on ice for 15 min. DNAs were added dropwise to COS cells, with the subsequent addition of 75 μl of 10 mM chloroquine. DNAs were left on cells for 16 to 24 h before the medium was changed. Cells were washed in PBS at 48 h posttransfection, and then 1 ml of Nonidet P-40 lysis buffer was added to each plate for 30 min on ice. Cells were then scraped off plates and placed in microcentrifuge tubes. The lysate was run through a 25-gauge syringe needle five times, and the cell debris was cleared by centrifugation. Ten microliters of the lysate was used in a luciferase assay to control for transfection efficiency.

Anti-LYL1 antibodies. A glutathione *S*-transferase (GST)–LYL1 fusion protein was constructed by cloning a *Hin*cII-*Hin*dIII fragment of the *LYL1* cDNA into the Klenow-blunted *Eco*RI site of the pGEX-2T vector (Pharmacia). Bacteria harboring the GST-LYL1 plasmid were grown to log phase and induced with IPTG (isopropyl-β-D-thiogalactopyranoside) at 37°C for 3 h. The GST-LYL1 fusion protein was purified from bacterial lysates by using glutathione agarose and SDS–10% PAGE and used for injections into rabbits (BAbCo). For the mouse monoclonal antibody, the same GST-LYL fusion protein was purified on glutathione-agarose and eluted with 5 mM glutathione, pH 8.0 (Sigma). Lymph node cells from immunized mice were fused with SP2/0 myeloma cells by established procedures. Hybridomas were screened by enzyme-linked immunosorbent assay using GST-LYL fusion protein, and those secreting antibodies with the desired properties were cloned and adapted to serum-free medium. Antibodies were precipitated from serum-free medium by using ammonium sulfate (310 g/liter) and resuspended in 1 M sodium borate buffer (pH 7.4) for subsequent use.

DNA binding and electrophoretic mobility shift analyses (EMSA). Nuclear extracts were prepared by the protocol of Schreiber et al. (43). Extracts were additionally passed through 0.2-μm-pore-size filters (Z-Spin; Gelman Sciences, Ann Arbor, Mich.) before storage at −70°C. One microgram of nuclear extract was incubated with a ^{32}P-labeled oligonucleotide (10,000 cpm) on ice for 15 min in a 15-μl binding reaction mixture containing 10 mM Tris (pH 7.5), 25 mM NaCl, 1 mM dithiothreitol, 6% glycerol, 16 ng of poly(dI-dC), and bovine serum albumin (BSA) fraction V to 20 μg of protein per reaction. Complexes were resolved by nondenaturing 6% polyacrylamide–0.25× Tris-borate-EDTA gel electrophoresis. Antibodies were incubated with the binding reaction mixture overnight on ice prior to adding the labeled probe. The sequence of the μE2 oligonucleotide was CCCAC<u>AGCAGGTGGC</u>AGGA; the sequence of the probe containing the LYL1-E12 selected site was GGACCTG<u>AACAGATGTT</u> TGGCT.

LYL1-E12 PCR-assisted site selection. A GST-LYL1 fusion protein containing the bHLH region was constructed by inserting a *Hin*dIII-*Eco*RI fragment of pSPLYLΔ2-128 into the *Eco*RI site of the pGEX-2T vector. A GST-LYL1 mutant lacking the HLH sequence was constructed by inserting an *Acc*I-*Hin*dIII fragment of pSP18ΔHLH into the *Sma*I site of the pGEX-2T vector. A maltose-binding protein (MBP)–E12 fusion protein was constructed by inserting a *Pst*I-*Hin*dIII fragment of E12 cDNA into the pMALc2 vector (New England BioLabs, Beverly, Mass.). Bacterial lysates containing fusion proteins were sonicated and

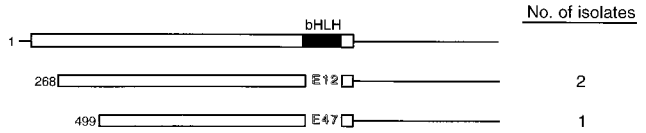

FIG. 1. Interaction of LYL1 and E2a proteins in yeast cells. Schematic illustrations of the E2a clones isolated by yeast two-hybrid screening based on their interactions with LYL1. Inserts from pACT library clones are shown with respect to the full-length E2a transcripts described by Nourse et al. (35a); the relative positions of 5′ ends of cDNA inserts are denoted in base pairs. Inserts also contained different portions of the 3′ untranslated region and poly(A) tails.

centrifuged to remove particulate debris and filtered through 0.2-μm-pore-size filters. Lysates containing LYL1 and E12 were mixed and incubated at 37°C for 45 min, and complexes were harvested on glutathione-agarose. The template oligonucleotide was a 55-mer containing 15 random internal nucleotides (CTCAGACGGATCCATTGCACN$_{15}$CTTCTAGATCTGTTCGCAGC). The *Bam*HI and *Bgl*II sites in the PCR primer arms of this oligonucleotide are underlined. Site selection was done as previously described for 10 rounds (29), except that the binding conditions were 20 mM Tris (pH 7.5), 25 mM NaCl, 10% glycerol, 25 μg of BSA, 25 μg of poly(dI-dC), 1 μg of oligonucleotide, and roughly 1 μg of LYL1-E12 heterodimer on 10 μl of glutathione-agarose in a final volume of 150 μl. For PCR amplification, aliquots were taken at either 10, 15, or 20 cycles. The material used for the following round was taken from the earliest time point at which a product was seen only for LYL1-E12, not LYL1ΔHLH-E12. The products were cloned into the *Bam*HI site of pBluescript SK− (Stratagene, La Jolla, Calif.), and 30 clones were sequenced and analyzed visually to determine a consensus site.

RESULTS

Identification of E2a proteins as dimerization partners for LYL1 by using the yeast two-hybrid interactional cloning system. Transcriptional regulation by the bHLH protein LYL1 likely requires dimerization partners that are themselves members of the bHLH family of proteins. To screen for human proteins able to interact with LYL1 in lymphocytes, a yeast two-hybrid cloning approach was employed (10, 18). A cDNA fragment containing the first 226 amino acids of *LYL1* was cloned in frame with the GAL4 DNA-binding domain in the yeast vector pAS1-CYH2 (12). Yeast strain Y190 harboring this plasmid was then transformed with the pACT human B-lymphocyte cDNA library (12) and subjected to a screening procedure to select for transformants containing potential LYL1-interacting proteins. Of four million colonies screened, 24 were found to grow on plates containing selective media with 50 mM 3-aminotriazole and to activate expression of a *HIS3-lacZ* reporter gene. Plasmid DNAs were isolated from His$^+$, blue colonies and retransformed into Y190; 12 were recovered and assessed for the specificities of their interactions with LYL1 by testing for potential interactions with other GAL4 fusion proteins, including GAL4-lamin (5). Plasmid DNAs were isolated from six independent His$^+$, blue colonies that lacked nonspecific interactions and used for subsequent analyses.

Nucleotide sequence analyses of these clones showed that cDNA inserts from three of the six encoded proteins were identical to previously reported products of the *E2a* gene, which codes for several related proteins, referred to as E12, E47, and ITF1, that arise by alternative RNA splicing events (19, 34, 35, 45). Clones were isolated for both the E12 and E47 or ITF1 versions of the E2a transcripts, and all contained the bHLH portions of the encoded proteins (Fig. 1). The three other clones which interacted specifically with LYL1 did not code for *E2a* gene products or other known HLH proteins and are not discussed further here.

The HLH motifs of LYL1 and E2a are required for their interactions in vitro. The interactions of LYL1 and E2a proteins were further studied by immunoprecipitation analyses of

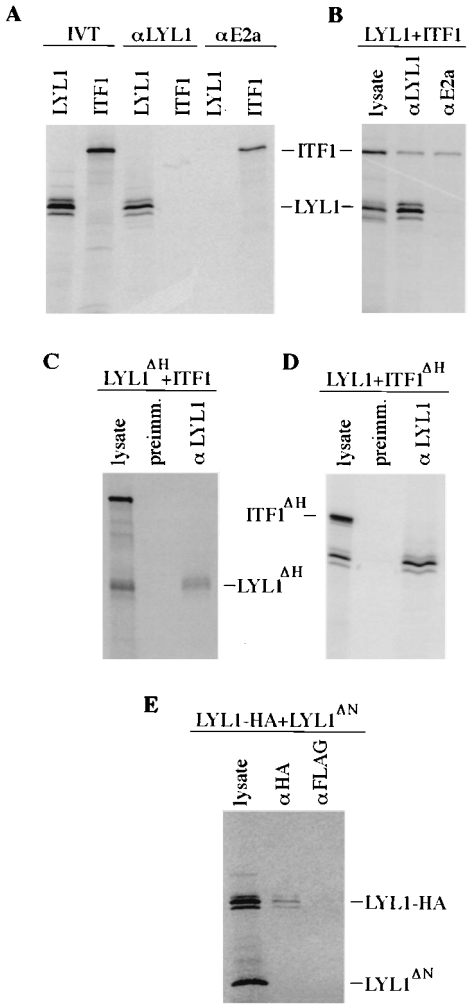

FIG. 2. In vitro interaction of LYL1 and E2a proteins. In vitro-translated (IVT) proteins were incubated with rabbit preimmune (preimm.), anti-E2a (αE2a), anti-LYL (αLYL1), or anti-HA (αHA) antibodies, and immunoprecipitates were analyzed by SDS-PAGE and PhosphorImager scanning. Proteins encoded by plasmids used to program translation reactions are indicated above each panel. Lanes marked "lysate" indicate translated proteins prior to immunoprecipitation analyses. The migrations of LYL1, LYL1$^{\Delta H}$, LYL1$^{\Delta N}$, ITF1, and ITF1$^{\Delta H}$ proteins are indicated on the right or left of panels. (A) Immunoprecipitations showing specificities of anti-LYL1 and anti-E2a antibodies. (B) Coprecipitation of LYL1 and E2a proteins. (C and D) LYL1-E2a interaction is dependent on HLH motifs. (E) LYL1 does not homodimerize in vitro. Anti-flag epitope (αFLAG) antibodies were used as a control.

in vitro-synthesized proteins. LYL1 and ITF1 were cotranslated in vitro with reticulocyte lysates and subsequently immunoprecipitated with an anti-LYL1 antiserum. The antiserum was specific for LYL1 and did not cross-react detectably with ITF1 (Fig. 2A). Under these conditions, approximately 25% of the ITF1 present in the reaction mixture coprecipitated with LYL1 (Fig. 2B). When an anti-E2a monoclonal antibody (25) was used for immunoprecipitation, a significantly smaller fraction of LYL1 was coprecipitated, suggesting that only a portion of input ITF1 was complexed with LYL1.

To establish whether the interaction of ITF1 with LYL1 was dependent upon the HLH motif of LYL1, a mutant lacking this region was assessed for its ability to coprecipitate ITF1. The LYL1$^{\Delta H}$ mutant was recognized and precipitated by the anti-LYL antiserum but was incapable of coprecipitating ITF1 (Fig.

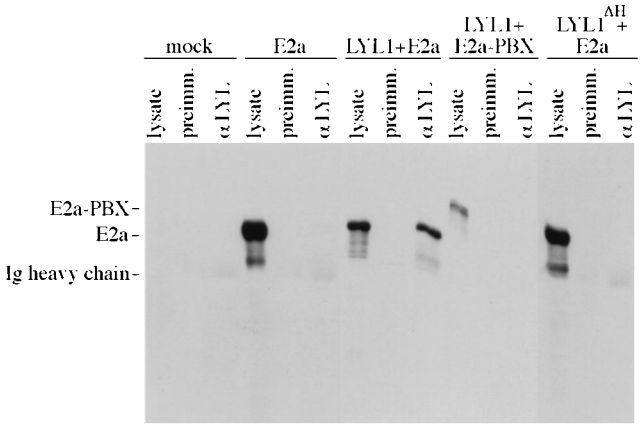

FIG. 3. In vivo association of LYL1 and E2a proteins in transfected MO-COS cells. Expression constructs for LYL1 and E2a (ITF1 clone) proteins were transfected singly or in combination into MO-COS cells. Transfected cells were harvested at 48 h, and total cellular lysates were incubated with preimmune (preimm.) or anti-LYL (αLYL) antibodies. Immunoprecipitated proteins were analyzed by Western blot analysis using an anti-E2a monoclonal antibody (25). The migrations of E2a and others are indicated on the left. Proteins encoded by constructs used for each transfection are indicated above the lanes. Lanes labeled "lysate" indicate cellular lysates from transfected cells prior to immunoprecipitation analysis demonstrating expression of E2a from transfected constructs. Ig, immunoglobulin.

2C). The interaction between ITF1 and LYL1 was also dependent upon the HLH of ITF1, as a mutant ITF1 lacking its bHLH motifs failed to coprecipitate with LYL1 (Fig. 2D). Similar results were obtained when E12 was employed in place of ITF1 (data not shown).

The potential of LYL1 to homodimerize with itself was also studied by using in vitro-synthesized proteins. For these studies, LYL1 was tagged at its carboxy terminus with the influenzavirus HA epitope (16). The epitope-tagged LYL1 was cotranslated in vitro with a mutant LYL1 protein ($LYL1^{\Delta N}$) containing a deletion of the 128 N-terminal amino acids, and immunoprecipitation was performed with an anti-HA monoclonal antibody. No $LYL1^{\Delta N}$ was coprecipitated with LYL1 (Fig. 2E), indicating that LYL1 is incapable of homodimerization under these experimental conditions. Thus, in vitro studies indicated that the HLH motif of LYL1 was capable of mediating specific heterotypic interactions with E2a proteins but incapable of homotypic interactions with itself. This conclusion is consistent with the fact that no clones encoding LYL1 were isolated in the yeast screening despite normal expression of LYL1 in B lymphocytes, the cell type from which the pACT library was prepared.

In vivo interaction of LYL1 and E2a proteins. The potential for LYL1 and E2a protein interactions was investigated in vivo by using transfected MO-COS cells. Under these conditions, cell staining showed LYL1 to be exclusively nuclear (data not shown). Immunoprecipitation analysis of cells cotransfected with LYL1 and ITF1 showed that ITF1 was present in anti-LYL immunoprecipitates, as detected by Western blot analysis of the precipitated proteins using the E2a monoclonal antibody (Fig. 3). No E2a proteins were detected in LYL immunoprecipitates of mock-transfected cells or cells transfected with ITF1 alone (Fig. 3). The in vivo interaction of LYL1 with ITF1 was dependent on the LYL1 HLH motif, as a mutant ($LYL1^{\Delta H}$) lacking this region was incapable of coprecipitating E2a from cotransfected MO-COS cells (Fig. 3). Similarly, the E2a-PBX1 fusion protein, which lacks the HLH of E2a, was not coprecipitated with LYL1 from cotransfected MO-COS cells (Fig. 3). These data confirmed the results of yeast and in vitro analyses, indicating that LYL1 and E2a proteins specifically interact with each other and that this interaction requires their HLH motifs.

Endogenous LYL1 proteins are present in a complex with E2a proteins. The compositions of potential endogenous cellular complexes containing LYL1 were investigated by immunoprecipitation analyses. By using specific antisera, LYL1 was precipitated from various cell lines expressing differing levels of LYL1 (Fig. 4A). This ranged from no detectable LYL1 RNA or protein in the Jurkat T-ALL line to low levels of LYL1 in the SUP-T7 cell line which carries a chromosomal translocation of the *LYL1* gene) and high levels of LYL1 in the KG-1 cell line (Fig. 4B). E2a proteins were coprecipitated with LYL1 only in lines containing LYL1 (SUP-T7 and KG-1), not in the Jurkat line, which does not express LYL1 (Fig. 4A). These data indicated that at least a portion of the endogenous LYL1 protein in hematolymphoid cells is present in a complex with E2a proteins, consistent with the above-mentioned results of in vivo and in vitro analyses demonstrating the dimerization potentials of these proteins. The reverse experiment to determine

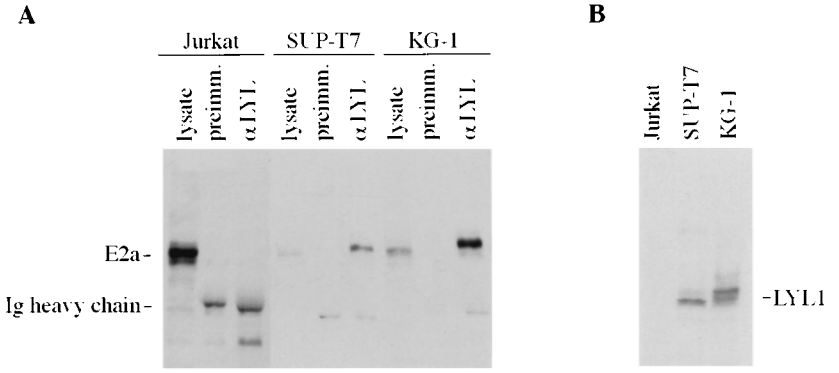

FIG. 4. Coprecipitation of endogenous LYL1 and E2a proteins from various hematolymphoid cell lines. (A) Cell lysates were incubated with preimmune (preimm.) or anti-LYL (αLYL) antibodies, and immunoprecipitated proteins were subjected to Western blot analyses using an anti-E2a monoclonal antibody (25). Cell lines employed for immunoprecipitations are indicated above. The migration of E2a protein is indicated on the left. Ig, immunoglobulin. (B) Total cellular lysates were analyzed by Western blot analyses using anti-LYL antibodies to demonstrate the presence or absence of endogenous LYL proteins in cells employed in panel A. The migration position of LYL1 protein is indicated on the right. The LYL1 migration differences in SUP-T7 and KG-1 cells are due to LYL1 truncation by chromosomal translocation in SUP-T7.

A

E12:LYL1:	N	A	A	C	A	G	A	T	G	T/g	T	N
%A	33	80	93	0	100	0	97	0	0	0	0	7
%C	3	7	3	100	0	0	0	0	0	0	7	47
%G	40	10	3	0	0	100	3	0	100	43	7	3
%T	23	0	0	0	0	0	0	100	0	57	87	43

B

E12:LYL1	A	A	C	A	G	A	T	G	T/g	T
E47:TAL1	A	A	C	A	G	A	T	G	G	T
E47:MYOD	N	A	C	A	C	C	T	G	T	N
E47:E47	N	G	C	A	G	G	T	G	T	N
μE2	A	G	C	A	G	G	T	G	G	C

FIG. 5. PCR-assisted site selection of a preferred LYL1-E12 DNA-binding site. (A) Heterodimers of GST-LYL1 and MBP-E12 fusion proteins were purified on glutathione-agarose and used for 10 rounds of selection. The resulting PCR products were cloned into pBluescript SK− (Stratagene). A total of 30 sites were sequenced and used to determine the percentages shown. (B) A comparison of the preferred LYL1-E12 DNA-binding site with other known bHLH DNA-binding sites. The canonical CA and TG residues are boxed. The E47 homodimer site was described by Sun and Baltimore (45). The E47-TAL1 site was described by Hsu et al. (21). The E47-MyoD site was described by Blackwell and Weintraub (7).

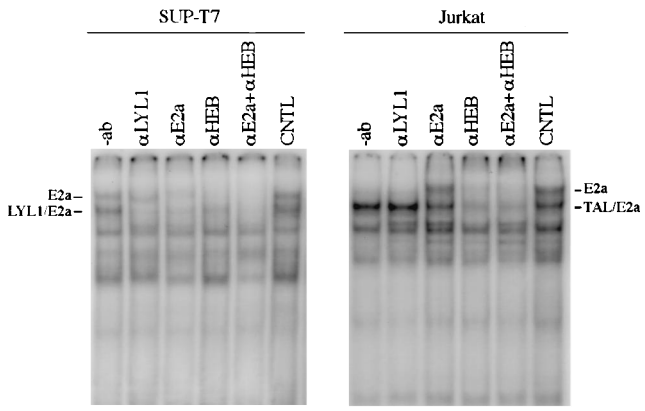

FIG. 6. EMSA showing endogenous LYL1 DNA-binding complexes in SUP-T7 cells, not Jurkat cells. LYL1-E2A DNA-binding complexes form in the SUP-T7 cell line, not the Jurkat cell line. For each cell line, 1 μg of nuclear extract was incubated with ^{32}P-labeled oligonucleotide containing the LYL1-E12 consensus site. Antibodies were preincubated with nuclear extracts (ab) before the addition of the oligonucleotide. The monoclonal anti-flag antibody (CNTL; IBI-Kodak, Rochester, N.Y.) was used as a negative control for antibody disruption studies. Monoclonal anti-E2a antibody (αE2a; Pharmingen, San Diego, Calif.) and polyclonal anti-HEB antibody (αHEB; Santa Cruz Biotechnology, Santa Cruz, Calif.) were used per instructions from suppliers. Gels were visualized by using PhosphorImager software.

the fraction of LYL1 complexed with E2a proteins was not successful because of the limitations of the antibody reagents employed for these studies.

DNA binding by LYL1-E2a heterodimeric complexes. The DNA-binding preferences of LYL1-E2a heterodimers were investigated by PCR-assisted site selection. Crude bacterial lysates of GST-LYL1 and MBP-E12 were mixed, and GST-LYL1-containing complexes were purified on glutathione-agarose. This purification allowed the preferential isolation of GST-LYL1 and MBP-E12 complexes over E12-E12 complexes, since the glutathione-agarose did not bind the MBP-E12 fusion protein (data not shown). Each round of site selection with LYL1-E12 was performed in parallel with LYL1$^{\Delta HLH}$-E12 as a negative control, since this LYL1 construct has no ability to bind E12 and therefore cannot form a DNA-binding heterodimer. After 10 rounds of selection, 29 independent LYL1-E12-selected sites were sequenced and yielded a clear consensus, 5′-AACAGATG(T/g)T-3′, as shown in Fig. 5A. The consensus consisted of two half sites, with the 5′ half, AACAG, similar or identical to half sites determined previously for E2a homo- or heterodimers. The 3′ half, ATG TT, was deduced to comprise a LYL1 half site and was notable for a strong preference for A in the core at position 6 of the overall consensus. The LYL1 half site is similar to that determined previously for TAL1 complexed with E47 (21). In fact, LYL1 and TAL1 are the only bHLH proteins shown to prefer an AT base pair over a CG base pair at this internal site (Fig. 5B). To flank the ATG half site, LYL1 demonstrated a slight preference for T over G, while TAL1 had the opposite, a slight preference for G over T at that position.

The LYL1-E12 consensus site was tested for its ability to bind endogenous proteins in nuclear extracts prepared from various hematolymphoid cell lines. SUP-T7, which ectopically expresses LYL1 because of a chromosomal translocation, contained a complex whose formation could be disrupted by anti-LYL or anti-E2a antibodies, confirming the presence of both LYL1 and E2a proteins. The same complex was partially disrupted by anti-HEB antibodies (which weakly cross-react with E2a proteins). Disruption of the LYL1-containing complex appeared to be more complete with a combination of E2a and HEB antibodies, suggesting that both serve as partners in SUP-T7 cells. In contrast, a complex present in Jurkat cells with a mobility similar to that of LYL1-E2a or LYL1-HEB complexes in SUP-T7 cells was almost completely disrupted by anti-E2a or anti-HEB antibodies but not anti-LYL antibodies, suggesting that it represents a TAL1-E2a complex, as reported by Hsu et al. (21) (Fig. 6). This interpretation is consistent with the expression of LYL1 in SUP-T7 cells, not Jurkat cells (Fig. 4), whereas TAL1 shows the opposite expression profile. LYL1-containing complexes that bound the consensus site were also detected in myeloid, mixed-lineage, and B-lymphocyte cell lines (Fig. 7A), all of which express LYL1 mRNA (17, 28, 31). No LYL1 complex was observed by EMSA in Molt4 cells, consistent with the general absence of LYL1 in T-lineage cells.

To assess the specificity of protein-DNA interaction, EMSA using a different E box site, the μE2 sequence from the immunoglobulin heavy-chain enhancer whose sequence is shown in Fig. 5B, was performed. This site differs from the preferred LYL1-E12 site at an internal core residue and in the residues flanking the core sequence and has been shown previously to be bound by E2a protein dimers (for a review, see reference 26). EMSA showed that complexes containing LYL1 or TAL displayed a strong preference for the LYL1 consensus site over the μE2 site (Fig. 7B). By comparison, complexes containing E2a but lacking LYL1 or TAL generally displayed the opposite, a preference for the μE2 site over the LYL1 consensus site. Thus, the sequence preferences detected in vitro by PCR-assisted site selection also appeared to be a feature of LYL1 DNA-binding complexes detected in nuclear extracts of cell lines representative of various hematopoietic lineages.

DISCUSSION

Our studies show that LYL1 has biochemical properties characteristic of class II HLH proteins. One of these properties is the capacity to form heterodimeric complexes with class I HLH proteins, as we demonstrated here for LYL1 under a variety of conditions. We initially searched for potential dimerization partners of LYL1 by yeast two-hybrid screening of a cDNA library constructed from B cells which typically express

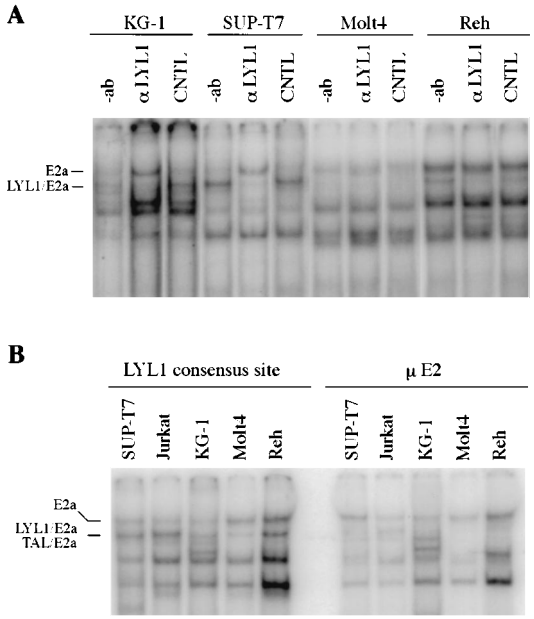

FIG. 7. Endogenous LYL1 DNA-binding complexes in various cell lines. (A) LYL1-E2A complexes in various human cell lines. KG-1 (myeloid), SUP-T7 (mixed-lineage T-ALL), Molt4 (T-lymphocyte), and Reh (pre-B-lymphocyte) cells were treated as described in the legend to Fig. 6. (B) LYL1-containing complexes can distinguish between the LYL1 consensus site and the μE2 site. One microgram of nuclear extract was incubated with either the LYL1 consensus site (AACAGATGTT) or the μE2 site (AGCAGGTGGC) for EMSA. Gels were visualized by using PhosphorImager software.

LYL1. The only HLH proteins identified by this approach were the widely expressed E2a proteins. Interactions between LYL1 and E2a proteins could be reconstituted in vitro or in transiently transfected cells and required the HLH motifs of both proteins. Most significantly, endogenous complexes containing LYL1 and E2a proteins were detected in several cell types, a T-ALL cell line ectopically expressing LYL1 because of chromosomal translocation and promonocytic and pre-B-cell lines. Conversely, homodimeric LYL1 complexes were not detected in vitro by immunoprecipitation or in vivo by EMSA.

As a heterodimeric complex with E2a proteins, LYL1 displayed a distinctive DNA-binding preference different from that exhibited by homodimeric class I HLH proteins. In fact, PCR-assisted site selection revealed a unique preference for an A nucleotide in the core of the LYL1 half site, a feature that appears to distinguish the LYL and TAL subfamily of class II HLH proteins. The consensus site preferred by E2a-LYL1 heterodimers is virtually identical to that determined for E47-TAL1, which is not surprising given the 89% identity between the bHLH regions of LYL1 and TAL1. The minor differences in these sites were found at position 9 flanking the ATG core, where TAL1 has been reported to show a slight preference for a G residue versus a slight T preference shown by LYL1. This could have resulted from differences in the methodology or protein substrates employed. Alternatively, the observed differences may represent the influence of other domains on DNA binding, since outside their bHLH domains LYL1 and TAL1 do not have significant homology.

EMSA of nuclear extracts from several cell types showed that the consensus site was recognized by endogenous LYL1-E2a protein complexes. LYL1-HEB complexes were also observed under these conditions. LYL1-containing heterodimers could distinguish between the consensus site and the μE2 site. The TAL-E2a complex in Jurkat extracts also showed a similar ability to distinguish between the two sites. This ability may contribute to specific recognition of binding sites in potential target genes for LYL1- or TAL-containing protein complexes. However, the functional relevance of the LYL1-preferred binding site is still unproved, as there have been no reports of in vivo DNA-binding sites activated by either LYL1 or TAL1. A search of the Eukaryotic Promoter Database (release 40) located some potential target genes whose regulatory regions contain LYL1-E2a consensus sites and are known to be expressed in the same tissue types as LYL1. Oncogenes c-*myb* and c-*fos* contain the consensus LYL1-E12 site. The consensus site is also present in the promoter regions of the human major histocompatibility complex II gene DRα, the δ-globin gene, and the pro-interleukin 1β gene. Further studies will determine whether any of these genes are actual targets of LYL1.

A biological role for LYL1 has not yet been reported, but our results indicate that this is likely to be mediated as heterodimers with class I HLH proteins, consistent with the paradigm established by studies of the myogenic HLH proteins. LYL1 mRNA is expressed in several hematopoietic lineages, with the exception of T lymphocytes. There is some overlap with TAL1 expression, particularly in erythroid and monocytic lineages, but LYL1 appears to be uniquely expressed in B lymphocytes (17, 28, 31, 47). Gene targeting studies have shown that the class I E2a proteins are essential for B-lymphoid development (4, 53) but not for other lineages, in spite of their widespread expression and known capacity to heterodimerize with various class II HLH proteins. These knockout studies suggest a special role in lymphoid development for E2a proteins which are subunits of B-cell-specific E2 box DNA-binding proteins. LYL1 is unlikely to be a component of the latter, given its strong preference for sites different from the immunoglobulin E boxes, thus the contributions of LYL1 to B-cell development in the context of E2a proteins remain to be defined. By comparison, functional studies implicate TAL1 as an erythroid differentiation factor. TAL1 has been detected in endogenous complexes with E2a proteins in erythroid cell lines. Forced overexpression of wild-type TAL1 increased the percentages of human and mouse erythroid precursor cell lines that spontaneously differentiated, while antisense TAL1 blocked the ability of the erythroid lines to be chemically induced to differentiate (1). Recently, gene targeting studies in mice demonstrated that TAL1 is essential for embryonic blood formation (37, 44). Furthermore, in adult chimeric mice, double knockout TAL1 null embryonic stem cells did not contribute to any of the hematopoietic cell populations, indicating that TAL1 is necessary for all hematopoiesis (36). Given the reported coexpression of LYL and TAL1 in many hematopoietic cells, these data support unique, nonoverlapping functions in spite of their similar DNA-binding properties, perhaps analogous to the myogenic HLH proteins.

As for a role in the development of T-ALL cells, an important consideration is the high (89%) identities of LYL1, TAL1, and TAL2 within their bHLH domains, whereas outside of these the proteins are not similar. Furthermore, some translocated alleles of TAL1 and TAL2 encode proteins with truncated amino termini, leaving only the bHLH domain and a short carboxy-terminal region of the proteins intact (2). Thus, the oncogenic requirements for this subfamily of HLH proteins in T-ALL cells may be limited to their bHLH motifs. Another shared property of these proteins is their ability to interact with RBTN proteins (46, 49). This family of LIM domain proteins, like TAL and LYL1 proteins, are ectopically expressed in T-ALL cells by chromosomal translocations. LIM domains are thought to mediate protein-protein interactions and are found

in a number of proteins implicated in developmental regulation (39). The RBTN-TAL1 interaction is mediated by the bHLH domain of TAL1 and the LIM domain of RBTN, and interactions are also seen with either LYL1 or TAL2, presumably also through their conserved bHLH motifs.

One possible role for the ectopic expression of LYL1 in T-ALL cells is the perturbation of the normal function of the bHLH proteins usually expressed in T cells. Since the E2a-LYL1 heterodimer has a different preferred binding site than does the E2a homodimer, ectopic LYL1 may redirect bHLH dimers to a subset of genes not normally active in T cells and activate gene transcription, leading to growth deregulation. Conversely, LYL1 could act as a negative regulator of genes controlled by E2a homodimers, since any E2a-LYL1 heterodimers that were formed would no longer bind to the E2a homodimer sites. Recently, an HEB-E2a complex has been identified on an E box site in the enhancer for the developmentally regulated *CD4* gene (41). Disruption of the expression levels of such T-cell markers could cause maturation arrest and lead to clonal expansion of the immature lymphocyte. Structure-function analyses are compromised by the fact that neither TAL1 nor LYL1 read out as oncoproteins in standard transformation assays, although coexpression of TAL1 with the v-*Abl* oncogene in a T-lymphocyte line decreases the latency of lymphoid tumors in a mouse model (14). Transgenic mouse models of TAL1 have produced conflicting results, with one report of nontransformation with the CD2 regulatory elements (38) and another report of lymphomas at low-level incidence (3 of 38 mice) in an independently generated mouse line with the proximal LCK promoter (27). These differences may reflect the levels of expression or genetic backgrounds of the mice.

The studies reported here do not address the potential transcriptional properties of LYL1 complexed with class I HLH proteins. On the basis of the MyoD paradigm and the fact that E2a proteins contain transcriptional activation domains, we predict that LYL1-E2a heterodimers should function as activator complexes through the consensus sites determined in our studies. In preliminary studies, LYL1 itself does not contain activator properties when fused to the heterologous GAL4 DNA-binding domain (31). This contrasts with reports that the amino terminus of TAL1, when fused to the GAL4 DNA-binding domain, is capable of transactivating a heterologous promoter (40). Furthermore, TAL1-E47 complexes have been shown to have modest transactivation potential on reporter genes containing multimers of the TAL-E2A site in fibroblasts in the presence of Id1 (23). Id1 completely inhibits transactivation by E47 homodimers but, under transient-transfection conditions, did not block DNA binding or transactivation by TAL1-E47 complexes. However, another report indicates that exogenous Id1 can interfere with endogenous TAL1-E2a DNA binding, suggesting that under some conditions, Id1 competes with TAL1 for E2a binding (48). These studies point out the complexities encountered when assessing the transcriptional properties of a given HLH complex which may be cell type specific and influenced by interactions with other HLH and non-HLH proteins. Our studies predict that the transcriptional roles of LYL1 are likely to be mediated as heterodimeric complexes with class I HLH proteins, and future studies are needed to establish the transcriptional properties of these complexes.

ACKNOWLEDGMENTS

This work was supported by NIH grants CA42971 and CA34233. A. M. was supported in part by a grant from the National Science Foundation and by PHS grant no. CA09302, awarded by the National Cancer Institute. L.N. was supported by a fellowship from the Howard Hughes Medical Institute. M.L.C. is a scholar of the Leukemia Society of America.

We gratefully acknowledge Steve Elledge, Stan Fields, Yakop Jacobs, Christian Nelson, and Tom Kadesch for reagents and advice.

REFERENCES

1. **Aplan, P. D., K. Nakahara, S. H. Orkin, and I. R. Kirsch.** 1992. The SCL gene product: a positive regulator of erythroid differentiation. EMBO J. **11:**4073–4081.
2. **Baer, R.** 1993. TAL1, TAL2, and LYL1: a family of basic helix-loop-helix proteins implicated in T cell acute leukemia. Semin. Cancer Biol. **4:**341–347.
3. **Bain, G., S. Gruenwald, and C. Murre.** 1993. E2A and E2-2 are subunits of B-cell-specific E2-box DNA-binding proteins. Mol. Cell. Biol. **13:**3522–3529.
4. **Bain, G., E. C. R. Maandag, D. J. Izon, D. Amsen, A. M. Kruisbeek, B. C. Weintraub, I. Krop, M. S. Schlissel, A. J. Feeney, M. Van Roon, M. Van Der Valk, H. P. J. Te Riele, A. Berns, and C. Murre.** 1994. E2a proteins are required for proper B cell development and initiation of immunoglobulin gene rearrangements. Cell **79:**885–892.
5. **Bartel, P., C.-T. Chien, R. Sternglanz, and S. Fields.** 1993. Elimination of false positives that arise in using the two-hybrid system. BioTechniques **14:**920–924.
6. **Begley, C. G., P. D. Aplan, S. M. Denning, B. F. Haynes, T. A. Waldmann, and I. R. Kirsch.** 1989. The gene SCL is expressed during early hematopoiesis and encodes a differentiation-related DNA-binding motif. Proc. Natl. Acad. Sci. USA **86:**10128–10132.
7. **Blackwell, T. K., and H. Weintraub.** 1990. Differences and similarities in DNA-binding preferences of MyoD and E2a protein complexes revealed by binding site selection. Science **250:**1104–1110.
8. **Breeden, L., and K. Nasmyth.** 1985. Regulation of the yeast HO gene. Cold Spring Harbor Symp. Quant. Biol. **50:**643–650.
9. **Chen, Q., J.-T. Cheng, L.-H. Tsai, N. Schneider, G. Buchanan, A. Carroll, W. Crist, B. Ozanne, M. J. Siciliano, and R. Baer.** 1990. The *tal* gene undergoes chromosome translocation in T cell leukemia and potentially encodes a helix-loop-helix protein. EMBO J. **9:**415–424.
10. **Chien, C.-T., P. L. Bartel, R. Sternglanz, and S. Fields.** 1991. The two-hybrid system: a method to identify and clone genes for proteins that interact with a protein of interest. Proc. Natl. Acad. Sci. USA **88:**9578–9582.
11. **Davis, R. L., P. F. Cheng, A. B. Lassar, and H. Weintraub.** 1990. The MyoD DNA binding domain contains a recognition code for muscle-specific gene activation. Cell **60:**733–746.
12. **Durfee, T., K. Becherer, P.-L. Chen, S.-H. Yeh, Y. Yang, A. E. Kilburn, W.-H. Lee, and S. J. Elledge.** 1993. The retinoblastoma protein associates with the protein phosphatase type 1 catalytic subunit. Genes Dev. **7:**555–569.
13. **Ellenberger, T., D. Fass, M. Arnaud, and S. C. Harrison.** 1994. Crystal structure of transcription factor E47: E-box recognition by a basic region helix-loop-helix dimer. Genes Dev. **8:**970–980.
14. **Elwood, N. J., A. R. Green, A. Melder, C. G. Begley, and N. Nicola.** 1994. The SCL protein displays cell-specific heterogeneity in size. Leukemia **8:**106–114.
15. **Ferre-D'armare, A. R., G. C. Prendergast, E. B. Ziff, and S. K. Burley.** 1993. Recognition by MAX of its cognate DNA through a dimeric b/HLH/Z domain. Nature (London) **363:**38–45.
16. **Field, J., J.-I. Nikawa, D. Broek, B. MacDonald, L. Rodgers, I. A. Wilson, R. A. Lerner, and M. Wigler.** 1988. Purification of a *RAS*-responsive adenylyl cyclase complex from *Saccharomyces cerevisiae* by use of an epitope addition method. Mol. Cell. Biol. **8:**2159–2165.
17. **Green, A. R., E. Salvaris, and C. G. Begley.** 1991. Erythroid expression of the 'helix-loop-helix' gene, SCL. Oncogene **6:**475–479.
18. **Harper, J. W., G. R. Adam, N. Wei, K. Keyomarsi, and S. J. Elledge.** 1993. The p21 Cdk-interacting protein CIP1 is a potent inhibitor of G1 cyclin-dependent kinases. Cell **75:**805–816.
19. **Henthorn, P., M. Kiledjian, and T. Kadesch.** 1990. Two distinct transcription factors that bind the immunoglobulin enhancer μE5/κE2 motif. Science **247:**467–470.
20. **Hsu, H.-L., J.-T. Cheng, Q. Chen, and R. Baer.** 1991. Enhancer-binding activity of the *tal-1* oncoprotein in association with the E47/E12 helix-loop-helix proteins. Mol. Cell. Biol. **11:**3037–3042.
21. **Hsu, H.-L., L. Huang, J. T. Tsan, W. Funk, W. E. Wright, J.-S. Hu, R. E. Kingston, and R. Baer.** 1994. Preferred sequences for DNA recognition by the TAL1 helix-loop-helix proteins. Mol. Cell. Biol. **14:**1256–1265.
22. **Hsu, H.-L., I. Wadman, and R. Baer.** 1994. Formation of *in vivo* complexes between the TAL1 and E2a polypeptides of leukemic T cells. Proc. Natl. Acad. Sci. USA **91:**3181–3185.
23. **Hsu, H.-L., I. Wadman, J. T. Tsan, and R. Baer.** 1994. Positive and negative transcriptional control by the TAL1 helix-loop-helix protein. Proc. Natl. Acad. Sci. USA **91:**5947–5951.
24. **Hu, J.-S., E. N. Olson, and R. E. Kingston.** 1992. HEB, a helix-loop-helix protein related to E2A and ITF2 that can modulate the DNA-binding ability of myogenic regulatory factors. Mol. Cell. Biol. **12:**1031–1042.
25. **Jacobs, Y., C. Vierra, and C. Nelson.** 1993. E2A expression, nuclear localization, and in vivo formation of DNA- and non-DNA-binding species during B-cell development. Mol. Cell. Biol. **13:**7321–7333.
26. **Kadesch, T.** 1992. Helix-loop-helix proteins in the regulation of immuno-

globulin gene transcription. Immunol. Today **13:**31–36.
27. **Kelliher, M. A., P. Leder, and D. C. Seldin.** 1995. TAL-1, a gene frequently activated in human T cell leukemia induces lymphomas in transgenic mice. Blood **86:**429a. (Abstract.)
28. **Kuo, S. S., J. D. Mellentin, N. G. Copeland, D. J. Gilbert, N. A. Jenkins, and M. L. Cleary.** 1991. Structure, chromosome mapping, and expression of the mouse Lyl-1 gene. Oncogene **6:**961–968.
29. **Lebrun, D. P., and M. L. Cleary.** 1994. Fusion with E2a alters the transcriptional properties of the homeodomain protein PBX1 in t(1;19) leukemias. Oncogene **9:**1641–1647.
30. **Mellentin, J. D., S. D. Smith, and M. L. Cleary.** 1989. *lyl-1*, a novel gene altered by chromosomal translocation in T cell leukemia, codes for a protein with a helix-loop-helix DNA binding motif. Cell **58:**77–84.
31. **Miyamoto, A., and M. L. Cleary.** Unpublished data.
32. **Monica, K., D. P. LeBrun, D. A. Dedera, R. Brown, and M. L. Cleary.** 1994. Transformation properties of the E2a-Pbx1 chimeric oncoprotein: fusion with E2a is essential, but the Pbx1 homeodomain is dispensable. Mol. Cell. Biol. **14:**8304–8314.
33. **Murre, C., P. S. Mccaw, and D. Baltimore.** 1989. A new DNA binding and dimerization motif in immunoglobulin enhancer binding, *daughterless*, *MyoD*, and *myc* proteins. Cell **56:**777–784.
34. **Murre, C., P. S. Mccaw, H. Vaessin, M. Caudy, L. Y. Jan, Y. N. Jan, C. V. Cabrera, J. N. Buskin, S. D. Hauschka, and A. I. Et.** 1989. Interactions between heterologous helix-loop-helix proteins generate complexes that bind specifically to a common DNA sequence. Cell **58:**537–544.
35. **Nelson, C., L. Shen, A. Meister, E. Fodor, and W. J. Rutter.** 1990. Pan: a transcriptional regulator that binds chymotrypsin, insulin, and AP-4 enhancer motifs. Genes Dev. **4:**1025–1043.
35a. **Nourse, J., J. D. Mellentin, N. Galili, J. Wilkinson, E. Stanbridge, S. D. Smith, and M. L. Cleary.** 1990. Chromosomal translocation t(1;19) results in synthesis of a homeobox fusion mRNA that codes for a potential chimeric transcription factor. Cell **60:**535–546.
36. **Porcher, C., W. Swat, K. Rockwell, Y. Fujiwara, R. Shivdasani, F. Alt, and S. H. Orkin.** 1995. Complete block to hematopoiesis in the absence of the T-cell leukemia oncoprotein, SCL/Tal-1. Blood **86:**253a. (Abstract.)
37. **Robb, L., I. Lyons, R. Li, L. Hartley, F. Kontgen, R. P. Harvey, D. Metcalf, and C. G. Begley.** 1995. Absence of yolk sac hematopoiesis from mice with a targeted disruption of the scl gene. Proc. Natl. Acad. Sci. USA **92:**7075–7079.
38. **Robb, L., J. E. J. Rasko, M. L. Bath, A. Strasser, and C. G. Begley.** 1995. Scl, a gene frequently activated in human t cell leukaemia, does not induce lymphomas in transgenic mice. Oncogene **10:**205–209.
39. **Sanchez-Garcia, I., and T. H. Rabbitts.** 1993. LIM domain proteins in leukemia and development. Semin. Cancer Biol. **4:**349–358.
40. **Sanchez-Garcia, I., and T. H. Rabbitts.** 1994. Transcriptional activation by TAL1 and FUS-CHOP proteins expressed in acute malignancies as a result of chromosomal abnormalities. Proc. Natl. Acad. Sci. USA **91:**7869–7873.
41. **Sawada, S., and D. R. Littman.** 1993. A heterodimer of HEB and an E12-related protein interacts with the CD4 enhancer and regulates its activity in T-cell lines. Mol. Cell. Biol. **13:**5620–5628.
42. **Schlissel, M., A. Voronova, and D. Baltimore.** 1991. Helix-loop-helix transcription factor E47 activates germ-line immunoglobulin heavy-chain gene transcription and rearrangement in a pre-T-cell line. Genes Dev. **5:**1367–1376.
43. **Schreiber, E., P. Matthias, M. M. Muller, and W. Schaffner.** 1989. Rapid detection of octamer binding proteins with 'mini-extracts," prepared from a small number of cells. Nucleic Acids Res. **17:**6419.
44. **Shivdasani, R. A., E. L. Mayer, and S. H. Orkin.** 1995. Absence of blood formation in mice lacking the t-cell leukaemia oncoprotein tal-1-scl. Nature (London) **373:**432–434.
45. **Sun, X., and D. Baltimore.** 1991. An inhibitory domain of E12 transcription factor prevents DNA binding in E12 homodimers but not in E12 heterodimers. Cell **64:**459–470.
46. **Valge-Archer, V. E., H. Osada, A. J. Warren, A. Forster, J. Li, R. Baer, and T. H. Rabbits.** 1994. The LIM protein RBTN2 and the basic helix-loop-helix protein TAL1 are present in a complex in erythroid cells. Proc. Natl. Acad. Sci. USA **91:**8617–8621.
47. **Visvader, J., C. G. Begley, and J. M. Adams.** 1991. Differential expression of the LYL, SCL and E2A helix-loop-helix genes within the hematopoietic system. Oncogene **6:**187–194.
48. **Voronova, A. F., and F. Lee.** 1994. The E2a and TAL-1 helix-loop-helix proteins associate in vivo and are modulated by Id proteins during interleukin 6-induced myeloid differentiation. Proc. Natl. Acad. Sci. USA **91:**5952–5956.
49. **Wadman, I., J. Li, R. O. Bash, A. Forster, H. Osada, T. H. Rabbitts, and R. Baer.** 1994. Specific *in vivo* association between the bHLH and LIM proteins implicated in human T cell leukemia. EMBO J. **13:**4831–4839.
50. **Weintraub, H., V. J. Dwarki, I. Verma, R. Davis, S. Hollenberg, L. Snider, A. Lassar, and S. J. Tapscott.** 1991. Muscle-specific transcriptional activation by MyoD. Genes Dev. **5:**1377–1386.
51. **Xia, Y., L. Brown, C. Y.-C. Yang, J. T. Tsan, M. J. Siciliano, R. I. Espinosa, M. M. Le Beau, and R. J. Baer.** 1991. TAL2, a helix-loop-helix gene activated by the (7;9)(q34;q32) translocation in human T-cell leukemia. Proc. Natl. Acad. Sci. USA **88:**11416–11420.
52. **Xia, Y., L.-Y. Hwang, M. H. Cobb, and R. Baer.** 1994. Products of the *tal2* oncogene in leukemic T cells: bHLH phosphoproteins with DNA-binding activity. Oncogene **9:**1437–1446.
53. **Zhuang, Y., P. Soriano, and H. Weintraub.** 1994. The helix-loop-helix gene E2a is required for B cell formation. Cell **79:**875–884.